PATENT
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	
Chris RUNDFELDT et al.	Group Art Unit: 1617
Application No.: 10/680,459	Examiner: D. R. CLAYTOR
Filed: October 6, 2003	Confirmation No.: 4494
For: USE OF DIHYDROIMIDAZOLONES FOR THE TREATMENT OF DOGS	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION UNDER 37 C.F.R. § 1.132

CHRIS RUNDFELDT hereby declares as follows:

1. I am the first named inventor of the above identified patent application. I am fully familiar with its content and its prosecution.

2. I submit this declaration as further evidence of the unobviousness of the compound referred to as "AWD 131-138" in treatment of canine idiopathic epilepsy.

3. I am aware of only two drugs which are effective against idiopathic epilepsy in dogs: phenobarbital and primidone. The structural formulae for these drugs are attached for convenience.

4. Phenobarbital and primidone are two of the oldest anticonvulsants known. They were developed, originally for use in humans. They are also effective in canines. While other drugs have been developed which are effective in humans, these newer drugs have not proven to be efficacious in canines.

5. The main reasons for the failure in dogs of drugs that are effective in humans are the high metabolic capacity of dogs, and their short gut length. To elaborate, these newer drugs do not achieve sufficient plasma levels to be effective anticonvulsants in canine idiopathic epilepsy.

6. The drug known both as AWD 131-138 and "imepitoin" has been shown to have good antiepileptic properties in dogs with idiopathic epilepsy. In view of the success with this drug, compounds with similar structures were tried. These are referred to as "AWD 131-139" and "AWD 131-175" respectively. Their chemical formulas are:

AWD 131-138: 1-(4-chlorophenyl)-4-(4-morpholinyl)-2,5-dihydro-1H-imidazol-2-one.

AWD 131-139: 1-(4-chlorophenyl)-4-piperidine-1-yl-2,5-dihydro-1H-imidazol-2-one.

AWD 131-175: 1-(4-chlorophenyl)-4-pyrrolidin-1-yl-2,5-dihydro-1H-imidazol-2-one.

The 3 dimensional structures of these 3 compounds are very similar, which suggested that they might function similarly. For convenience, the structures of these 3 compounds are attached.

7. As background, I point out that in experiments I either carried out myself or had carried out under my supervision, all three of these compounds were shown to follow the same mechanism of action in that they act as partial low affinity agonists at the benzodiazepine receptor. While the affinity to the receptor was mostly identical for AWD 131-138 and AWD 131-175, it was about 3 times higher for AWD 131-139. Further more, the compounds were effective in rodent models of induced seizures. As will be shown in the material which follows, however, the efficacy in rodents was not extrapolatable to canines.

8. It is important, indeed critical, in the treatment of canine idiopathic epilepsy, that the subject animal receive as constant an exposure to the drug as possible. This is because seizures can, and do occur at all times of the day, and at any day. For reasons that have been set forth in the declarations that have been filed previously, the unique metabolism and physiology of dogs has made this requirement problematic for drugs which are effective in other species.

9. The three compounds discussed supra were those tested to evaluate their metabolic stability and their pharmokinetics in canines.

10. In a first set of experiments, the rate at which the drugs were degraded was studied.

11. Microsomes were prepared from fresh dog livers, using standard methodologies. Protein concentrations, and total cytochrome P450 content were determined, again following standard methods.

12. The test substances were then incubated with the canine hepatic microsomes, at a concentration of test compound that was adjusted, in accordance with the individual solubility, to 70 µM for AED 131-138, 20 µM for AWD 131-139 and 5.3 µM for AWD 131-175. Due to the nature enzyme kinetics underlying metabolic reactions, the starting concentration of the test compound does not influence the reaction speed. However, higher concentrations are more easily analyzed. The mixture was pre-incubated, for 5 minutes, and then NADPH was added to start an NADPH-regenerating system. The reaction was allowed to proceed for six hours, for AWD 131-138 and AWD 131-175, and for three hours for AWD 131-139. The shorter time frame for AWD 131-139 was necessary in view of its rapid degradation. The reactions were stopped, and supernatants were analyzed. The extent of degradation was determined by comparing ultraviolet detector peak areas of test compounds with a control. The results are presented, in terms of the percentage of peak area remaining at the given time period.

Dog liver microsomes	Percentage of peak area remaining
Inactivated protein sample (control)	100%
AWD 131-138	
Active sample, 6 h incubation	82.9%
AWD 131-139	
Active sample a, 3 h incubation	44%
Active sample b, 3 h incubation	42%
AWD 131-175	
Active sample, 6 h incubation	33%

13. AWD 131-138 was the most stable compound, as will be seen from the table above. AWD 131-139 was degraded so rapidly that measurements were taken after only 3 hours. AWD 131-175 was metabolized very rapidly, with 67% having been degraded within 6 hours.

14. This in vitro study suggested that AWD 131-138 would be effective while the other two, structurally similar compounds, would not be. The rapid degradation of AWD 131-175 in dog liver microsomes, which is indicative of a very rapid metabolism in vivo, and the poor solubility of AWD 131-175, which is indicative of a low intestinal absorption, precluded its use in in vivo experiments; however, AWD 131-138 and AWD 131-139 were tested, as follows, to study their pharmacokinetics.

15. A single dose study was carried out on beagles, using AWD 131-138. Subject animals received an oral dose of the compound, at 20 mg/kg.

Blood samples were collected from the animals prior to dosing, and at .5, 1, 2, 4, 6, 8, 10, 12, and 24 hours after administration.

16. For AWD 131-139, a larger single dose (30 mg/kg) was administered and blood samples were taken as described for AWD 131-138. The higher dose was selected since, based on the in vitro dog hepatocytes metabolism data, we expected a lower maximal plasma level and a more rapid elimination of compound from plasma. Sufficiently high plasma levels are needed to accurately determine the AUC and were obtained with this high dose. For comparison only, the Cmax and AUC values for AWD 131-138 were also scaled to a fictive dose of 30 mg/kg using a factor of 1.5. This is a standard approach used in pharmacokinetics.

17. Blood samples from animals which had received AWD 131-138, and animals which had received AWD 131-139 were Na-heparinized, and analyzed via a validated, HPLC-MS/MS method.

C_{max} and area under the curve ("AUC") values, are presented:

Compound	C_{max} (ng/ml)	AUC (ng*h/ml)
AWD 131-138 (both genders)	5705 ng	26029
AWD 131-138 (scaled to 30 mg/kg)	8558 ng	39044
AWD 131-139 (male)	2029	3570
AWD 131-139 (female)	2983	6044

18. When one compares the single dose kinetic data obtained for AWD 131-138 and AWD 131-139, differences are evident which could not be predicted from the structures, or from the rodent data. To elaborate, maximal concentration for AWD 131-139 was only one third of that of AWD 131-138. This is coupled to the fact that the AUC, which represents exposure was much lower for AWD 131-139 than AWD 131-138. Further more, there was an obvious gender difference for AWD 131-139 in that male animals had even lower C_{max} and AUC values as compared to female animals. No such difference is known for AWD 131-138.

19. In the rodent models referred to supra, anticonvulsant activity was assessed at peak plasma exposure. Notwithstanding the fact that the maximal level of AWD 131-138 was about 3 times higher than that of AWD 131-139, their activity was similar in seizure models. The affinity of AWD 131-139 for the benzodiazepine receptor was about 3 times higher than that of AWD 131-138, which would compensate for the lower plasma levels.

20. In contrast to the results in the rodent models, one has the canine data. Here, C_{max} represents peak plasma levels, and AUC measures exposure over time. The AUC for AWD 131-139 was about 11 fold lower in male dogs and about 6.5 fold lower in female dogs than that of AWD 131-138, which indicates that the necessary degree of exposure for an anti-epileptic drug cannot be reached with AWD 131-139.

21. The results discussed in "20" validate the microsome studies, because the rapid metabolism of AWD 131-139 by the canine hepatic microsomes results in a greatly reduced AUC. A low AUC in turn, indicates that therapeutic plasma levels of the drug cannot be reached.

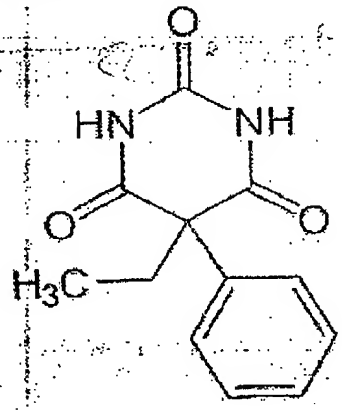
22. In conclusion, while the 3 drugs tested are very similar in structure, as well as in efficacy in rodent anti-convulsant models, only AWD 131-138, the subject of the patent application, provides a level of plasma exposure sufficient for treatment of canine idiopathic epilepsy.

23. I hereby declare that all statements made herein where done on the basis of my best knowledge and that all statements made are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

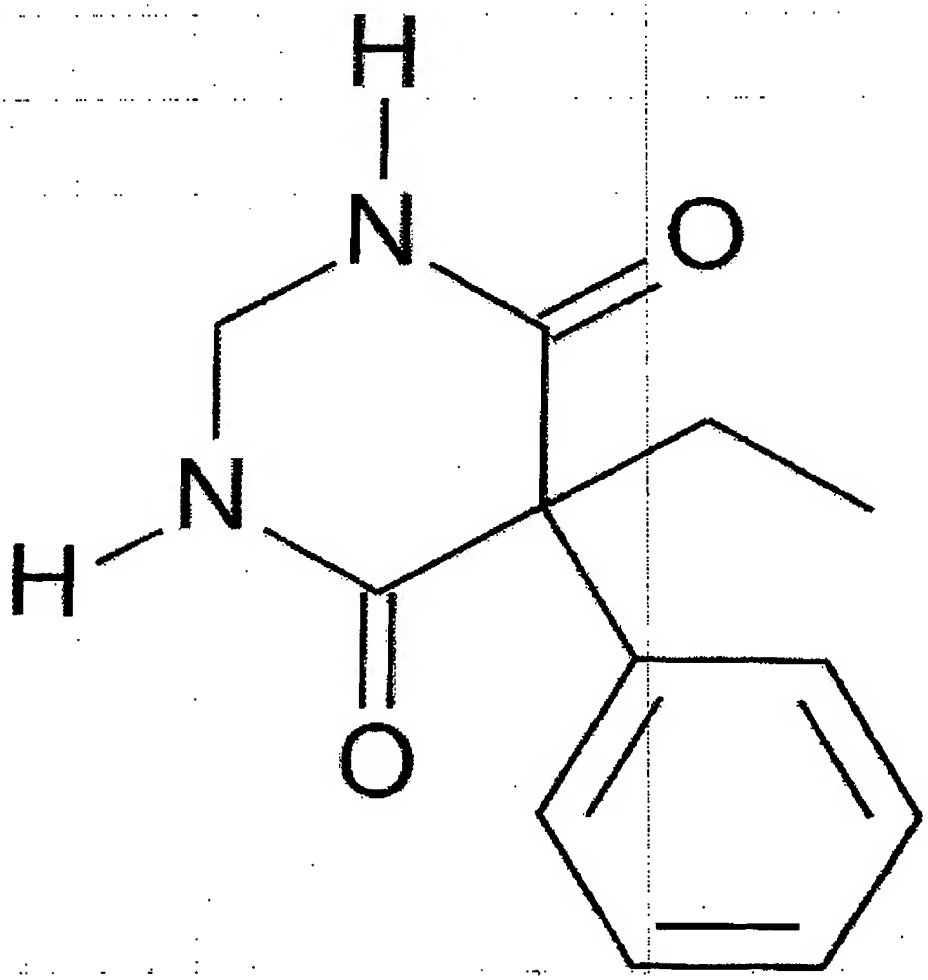
Dated: By: _____
PD Dr. Chris RUNDFELDT

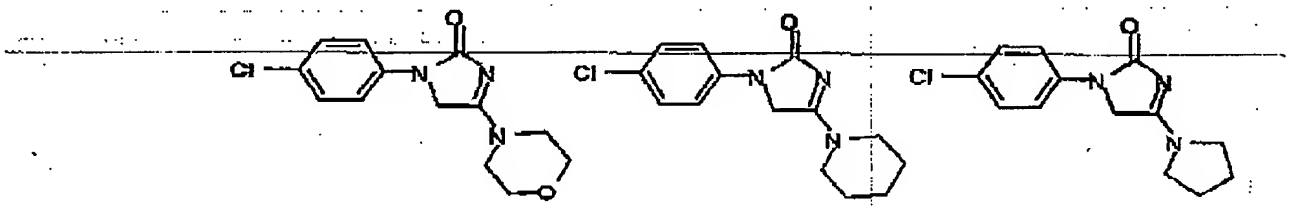
Attachments: Structural Formulas for Phenobarbital and primidone
Structural Formulas for AWD 131-138; AWD 131-139; AWD 131-175

File:Phenobarbital.svg



File:Primidone structure.svg





AWD 131-138 AWD 131-139 AWD 131-175